Interleukin-10 Modulates the Severity of Hypersensitivity Pneumonitis in Mice

Gunnar Gudmundsson, Assumpció Bosch, Beverly L. Davidson, Daniel J. Berg, and Gary W. Hunninghake

Department of Medicine, University of Iowa College of Medicine, and Veterans Administrations Medical Center, Iowa City, Iowa

Hypersensitivity pneumonitis (HP) is an inflammatory lung disease characterized by granuloma formation. We recently showed that interferon-γ (IFN-γ) is essential for inflammation and granuloma formation in HP. Interleukin-10 (IL-10) counteracts many of the biologic effects of IFN-γ, suggesting that IL-10 modulates inflammation and granuloma formation in HP. We compared the expression of HP in C57BL/6 mice that lack IL-10 (IL-10 knockout [KO]) with that in wild-type (WT) littermates. IL-10 KO and WT mice were exposed to the thermophilic bacteria *Saccharopolyspora rectivirgula* or to saline alone for 3 wk. The IL-10 KO mice had higher cell counts in their bronchoalveolar lavage fluid ($2.85 \pm 0.43 \times 10^6$) than did WT mice ($1.4 \pm 0.3 \times 10^6$/ml; $P < 0.03$), with a more prominent neutrophil response. They also had greater inflammation after antigen exposure than did the WT mice ($P < 0.0001$). There was increased upregulation of IFN-γ, IL-1, and tumor necrosis factor-α (TNF-α) mRNAs in the lungs of IL-10 KO mice. Adenovirus-mediated gene transfer of IL-10 to the liver of IL-10 KO mice reduced the inflammation from that seen in WT mice. These studies show that IL-10 has important anti-inflammatory properties in HP, and that lack of this cytokine leads to a more severe granulomatous inflammatory response. **Gudmundsson, G., A. Bosch, B. L. Davidson, D. J. Berg, and G. W. Hunninghake. 1998. Interleukin-10 modulates the severity of hypersensitivity pneumonitis in mice. Am. J. Respir. Cell Mol. Biol. 19:812–818.**

Hypersensitivity pneumonitis (HP) is caused by sensitization to and repeated inhalation of various organic antigens (1–8). The most common of these antigens are thermophilic actinomycetes that cause farmer's lung disease (2). Repeated exposures lead to granuloma formation in the lung and, in some patients, progression to end-stage lung disease with respiratory failure and cor pulmonale (1, 2, 8). Both immune-complex–mediated immunity and cell-mediated immunity are thought to be important for the pathogenesis of HP (9–11). The first response to antigen is an increase in polymorphonuclear leukocytes (PMN) in the alveoli and small airways (12–14). This is followed by an influx of mononuclear cells and formation of granulomas (1).

Mice exposed to the actinomycete *Saccharopolyspora rectivirgula* (SR; previous names *Micropolyspora faeni* and *Faenia rectivirgula*) or other thermophilic bacteria develop diffuse bronchoalveolitis and granulomas in the lung (15–21). With this murine model, we have previously shown that interferon-γ (IFN-γ) is essential for granuloma formation (21). Interleukin-10 (IL-10) is an important anti-inflammatory cytokine that can inhibit the production of many proinflammatory cytokines, including IFN-γ, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) (22–24). Both human and animal studies have shown that these proinflammatory cytokines play an important role in the pathogenesis of HP and other granulomatous lung diseases (26–31).

On the basis of these observations, we hypothesized that IL-10 is important for modulation of the inflammatory response in HP, and that lack of IL-10 would lead to a more severe inflammatory response and granuloma formation. No studies have previously evaluated the role of IL-10 in HP.

Materials and Methods

Animals

C57BL/6 mice with a targeted disruption of the IL-10 gene (replacement of a 500-bp fragment containing codons 5 to 55 of the first exon by a linker providing a termination codon, by a neo expression cassette, and by introduction

(*Received in original form August 25, 1997 and in revised form March 19, 1998*)

Address correspondence to: Gunnar Gudmundsson, University of Iowa College of Medicine GH C-321, 200 Hawkins Drive, Iowa City, IA 52242. E-mail: gunnar-gudmundsson@uiowa.edu

Abbreviations: complementary DNA, cDNA; conditioned medium, CM; hypersensitivity pneumonitis, HP; interferon-γ, IFN-γ; interleukin-10, IL-10; lipopolysaccharide, LPS; polymerase chain reaction, PCR; tumor necrosis factor-α, TNA-α.

Am. J. Respir. Cell Mol. Biol. Vol. 19, pp. 812–818, 1998
Internet address: www.atsjournals.org

of a termination codon into exon 3) (32) and wild-type (WT) littermates were purchased from Jackson Laboratories (Bar Harbor, ME). They were bred and raised in the Animal Care Facility at the University of Iowa. Female mice aged 8 to 11 wk were used for these studies. Previous studies have shown that at this age the IL-10 knockout (KO) mice do not have any significant pathologic findings in their lungs (32). The mouse were housed in an antigen-free and virus-free environment, and were maintained with standard mouse chow and water *ad libitum*. All procedures used in the study were in compliance with Animal Welfare Act Regulations and with the *Guide for the Care and Use of Laboratory Animals* (33).

Antigen

Antigen was prepared from a strain of SR obtained from the American Type Culture Collection (Catalog No. 29034; ATCC, Rockville, MD). It was grown in a trypticase soy broth in a shaking incubator at 55°C for 4 d, centrifuged, and washed three times with distilled water. The washed culture was then homogenized and lyophilized. Antigen was resuspended in pyrogen-free saline. A *Limulus* amebocyte lysate assay (Sigma Chemical Co., St. Louis, MO) showed that this material contained less than 20 ng/ml of endotoxin.

Induction of HP

HP was induced by instilling intranasally and under light anesthesia 150 μg of SR antigen in saline, as previously described (21). The material was applied at the tip of the nose and inhaled involuntarily. This was done for three consecutive days per week for 3 wk. We found that this was the optimal timing and dosing schedule (21). Previous studies have shown that 15 to 60% of the administered antigen reaches the lung under these conditions (34). Mice were killed with pentobarbital injection 4 d after the last antigen exposure.

Bronchoalveolar Lavage

After euthanasia, a 20-gauge catheter was inserted into the trachea. Samples of bronchoalveolar lavage fluid (BALF) were obtained by washing the lungs with three 1-ml aliquots of 0.9% saline. After centrifugation, BALF cell pellets were washed and resuspended in Hanks' balanced salt solution (HBSS), and total cell counts were made with a Coulter counter (Coulter Electronics, Hialeah, FL). Cytospin preparations were fixed and stained with Diff-Quik stain (Baxter, McGaw Park, IL). Differential counts were made on 200 cells, using standard morphologic criteria to identify the cells as neutrophils, eosinophils, lymphocytes, or macrophages.

Histologic Evaluation

Lungs were perfused with 2% paraformaldehyde through the heart and trachea, and were fixed in 2% paraformaldehyde–phosphate-buffered saline (PBS). The sections were embedded in paraffin, cut in 5-μm-thick sections and stained with hematoxylin and eosin (H&E). The sections were evaluated with light microscopy. The slides were evaluated without knowledge of the type of mouse or antigen exposure. The lung fields were evaluated for extent of inflammation and granuloma formation, and this was expressed as a percentage of the total area of the lung fields. The area covered by an eyepiece grid (0.99 × 0.99 mm, using ×100 magnification) was judged to be normal or abnormal. An average of 200 fields was evaluated for each mouse (17, 18, 21). The results of evaluation of the inflammatory changes were reproducible for two evaluations (r = 0.927).

Northern Blot Analysis

Total lung RNA was prepared using the guanidine isothiocyanate extraction and cesium chloride centrifugation method of Chirgwin and colleagues (35), as modified by Maniatis and coworkers (36). The RNA was fractionated on 1.5% agarose gels containing 2.2 M formaldehyde by the method of Lehrach and coworkers (37). The gels were stained with ethidium bromide and destained overnight in 0.1% ammonium acetate to assess RNA integrity and equivalent loading. *Escherichia coli* 23S and 16S messenger RNA (mRNA) served as standards. Subsequently, the RNA was transferred to Gene Screen Plus (NEN Research Products, Boston, MA), following the manufacturer's specifications, and was then crosslinked to the nylon membrane by exposure to UV light. Fixed membranes were prehybridized for 12 to 24 h at 42°C in a solution of 50% formamide, 1 M NaCl, 1× Denhardt's solution, 0.05 M Tris (hydroxymethyl) aminomethane (Tris), 1% sodium dodecyl sulfate (SDS), and 10% dextran sulfate. The membranes were hybridized in fresh solution at 42°C with ^{32}P-labeled complementary DNA (cDNA) probes for murine IFN-γ, TNF-α, or IL-1 obtained from Clontech (Palo Alto, CA). After hybridization, the filters were washed twice at room temperature in 1× standard saline citrate (SSC), followed by two rinses at 65°C in 1× SSC–1% SDS and a final room-temperature wash in 0.1× SSC. Hybridized membranes were then exposed to radiographic film.

Generation of Recombinant Adenovirus Expressing Human IL-10

Human IL10 (hIL-10) cDNA was obtained by polymerase chain reaction (PCR) with 5′ and 3′ flanking primers (5′-hIL-10*Bam*HI: 5′-CGCGGATCCCATGCACAGCTCAGCACTG-3′; 3′-hIL-10*Bam*HI: 5′-CGCGGATCCGCCACCCTGATGTCTCAGT-3′), using the clone pSRαhIL-10 as template (kindly provided by E. Field, University of Iowa). The PCR product was cloned, using the *Bam*HI restriction-site tails added to the oligonucleotide sequences, in a shuttle plasmid (pAdRSV4; 31). This shuttle plasmid contains the Rous sarcoma virus promoter, the SV40-polyA signal, and the genomic adenoviral sequences from 0 to 1 and 9 to 16 map units of human adenovirus type 5. Recombinant adenovirus expressing IL-10 was generated by homologous recombination between pAdRSVhIL-10 and human adenovirus serotype 5 derivative d1309, using standard methods (37). AdRSVLacZ has the same viral backbone as AdRSVhIL-10, and has been described previously (37). AdRSVLacZ expresses the gene for *E. coli* β-galactosidase.

In Vitro Evaluation of hIL-10 Bioactivity after AdRSVhIL-10 Gene Transfer

An *in vitro* functional assay for hIL-10 was performed to test the activity of AdRSVhIL-10, on the basis of the ability of IL-10 to inhibit the synthesis of IFN-γ by lectin-stimulated spleen cells. Six-week-old C57Bl/6 splenocytes were isolated with a Ficoll gradient (Pharmacia LKB, Piscataway, NJ), and were incubated for 48 h at 37°C under 5% CO_2 in RPMI 1640-10 complete medium, with dilutions of conditioned medium (CM) from HeLa cells previously infected with AdRSVhIL-10. Final concentrations of 4 μg/ml of concanavalin A (Con A) (Sigma) to stimulate spleen cells, and 2 ng/ml of recombinant hIL-2 (Genzyme; Cambridge, MA) to enhance the IFN-γ synthesis, were added at the same time. Untreated cells and CM harvested from cells transduced with AdRSVLacZ were used as negative controls. Different concentrations of recombinant hIL-10 were added as positive controls. All conditions were assayed in duplicate. Mouse IFN-γ levels were determined with the Intertest Mouse IFN-γ enzyme-linked immunosorbent assay (ELISA) kit (Genzyme). Bioactivity of hIL-10 is described as a 10 to 30% inhibition of IFN-γ synthesis compared with untreated controls (39).

In Vivo Evaluation of hIL-10 Bioactivity after AdRSVhIL-10 Gene Transfer to IL-10 KO Mice

Recombinant hIL-10 has previously been shown to protect IL-10 KO mice from lipopolysaccharide (LPS)-induced shock (40). To test the bioactivity of AdRSVIL-10 *in vivo*, we injected IL-10 KO mice with 5×10^8 pfu of AdRSVhIL-10 or AdRSVLacZ via a tail vein. Six days later, hIL-10 levels in blood were measured with the ELISA (Genzyme) mentioned previously. All mice were challenged with a lethal dose of LPS (10 μg) injected into the tail vein. Animals were monitored for survival during the following 72 h.

In a separate experiment, groups of IL-10 KO mice were injected in the tail vein with AdRSVhIL-10 or AdRSVLacZ to test for effects in the HP model. They were given 1×10^9 pfu of virus in 100 μl of PBS with 3% sucrose. Control animals received injections of PBS. Intranasal exposures to the SR antigen or saline were started 7 d after the tail vein injections, after hIL-10 production had been confirmed by measurement of hIL-10 in the blood of the mice.

Statistics

Statistical analysis was done with an unpaired (two-tailed) *t* test. Values are expressed as means ± SEM. Values of $P < 0.05$ were considered significant. Comparisons of histologic evaluations were done with one-way analysis of variance (ANOVA), with Tukey's procedure for multiple comparisons. Probit transformation was done on percent data, with the probit value of 0.1% replacing 0%.

Results

Bronchoalveolar Lavage

Exposure to antigen led to an increase in the total numbers of cells in BALF from both IL-10 KO and WT mice, as shown in Table 1. However, the IL-10 mice had significantly higher total cell counts in their BALF ($2.85 \pm 0.43 \times 10^6$/ml) than did the WT mice ($1.4 \pm 0.3 \times 10^6$). This was a significant difference ($P < 0.03$). Table 1 also shows the differential cell counts and demonstrates that both IL-10 KO and WT mice had increases in lymphocytes (60-fold and 35-fold, respectively). Also notable was the difference between the neutrophil response in the antigen-exposed IL-10 KO mice and that of the WT mice ($P < 0.01$). IL-10 KO mice had a 140-fold increase in neutrophils in their BALF whereas neutrophil influx in the WT mice was modest. These studies show that IL-10 modulates the cellular composition of BALF in HP.

Histology

We have previously shown that antigen exposure results in an inflammatory response and granuloma formation in WT mice (21). Figure 1 shows that the severity of the inflammatory response and granuloma formation are greater in IL-10 KO mice. Saline-exposed WT or IL-10 KO mice had no evidence of inflammation or granulomas. The cross-sectional area of lung involved in granulomatous inflammation after antigen exposure was significantly greater in the IL-10 KO mice (76.1%, mean − SEM = 73.1%, mean + SEM = 79%) than in the WT mice (46.6%, mean − SEM = 42.9%, mean + SEM = 50.4%), as shown in Figure 1 ($P < 0.0001$). This percentage underestimates the severity of inflammation in the IL-10 KO mice, because involved areas had more dense inflammation in these mice than in the WT mice (Figure 2). There is a potential for sampling errors with this method. For this reason, we examined a large number of fields ($n = 200$ in each animal). These

TABLE 1
Comparison of cells in BALF *

	Total Cells	Macrophages†	Neutrophils†	Lymphocytes†	Eosinophils†
IL-10 KO, saline-exposed	4.5 ± 1.2	4.3 ± 0.05 (97)	0.1 ± 0.04 (2)	0.1 ± 0.04 (1)	0.0 ± 0.0 (0)
IL-10 KO, antigen-exposed	28 ± 4.3‡§	8.0 ± 1.1 (28)	14 ± 1.4‡§ (49)	6.0 ± 1.0‡ (21)	0.6 ± 0.1‡ (2)
WT, saline-exposed	3.0 ± 1.0	2.9 ± 0.1 (99)	0.0 ± 0.0 (0)	0.1 ± 0.04 (1)	0.0 ± 0.0 (0)
WT, antigen-exposed	14 ± 3.0‡	9.5 ± 0.9‡ (68)	0.3 ± 0.1‡ (2)	3.5 ± 0.9 (25)	0.7 ± 0.3‡ (5)

*Cells × 10^5.
†Parentheses indicate percentage of cells.
There was no significant difference between the saline-treated IL-10 KO and the WT mice (for all cell types).
‡$P < 0.05$ for SR- versus saline-treated animals.
§$P < 0.05$ for IL-10 KO versus WT mice treated with SR.

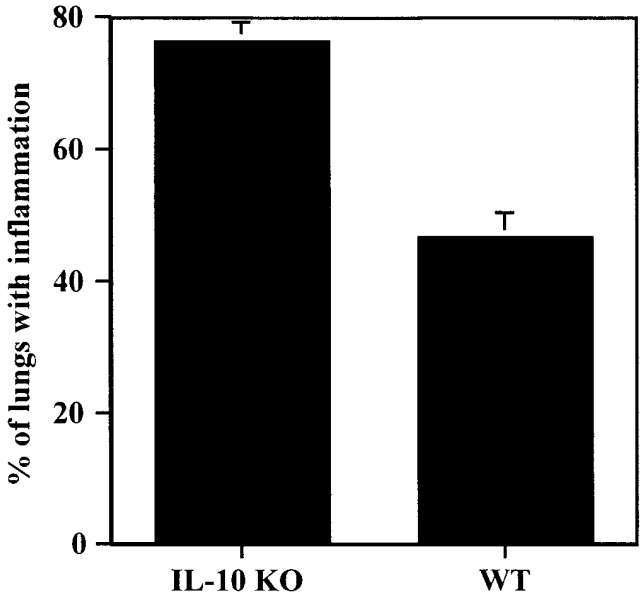


Figure 1. Quantitation of inflammation and granulomas in WT and IL-10 KO mice. IL-10 KO and WT mice were exposed to saline alone or to antigen, as described in MATERIALS AND METHODS. Amounts of inflammation and granulomas were quantitated as described in MATERIALS AND METHODS. Antigen-exposed IL-10 KO mice had significantly more inflammation than antigen-exposed WT mice ($P < 0.0001$). Data are expressed as means ± SEM for $n = 4$ to 6 animals in each group.

studies show that IL-10 modulates the severity of inflammation and granuloma formation in HP.

Proinflammatory Cytokine Expression

It has been suggested that IFN-γ, TNF-α, and IL-1 are necessary for granuloma formation in other models of granulomatous lung disease (25–31). Amounts of these cytokines in lungs are too low for measurement of their protein. Therefore, we measured mRNA levels for these cytokines in lung homogenates. When exposed to the SR antigen, the IL-10 KO mice had qualitatively more of the mRNAs for IFN-γ, IL-1, and TNF-α in lung homogenates (Figure 3) than did the WT mice. These observations suggest that IL-10 modulates the severity of HP by dampening the upregulation of cytokines that mediate inflammation and granuloma formation.

Human IL-10 Adenovirus

To confirm that the more severe response in IL-10 KO mice was due to the absence of IL-10 and not to another developmental process, we constructed a recombinant adenovirus vector expressing IL-10 for IL-10 replacement. The hIL-10–expressing adenovirus was tested for its bioactivity with an *in vitro* assay based on the ability of IL-10 to inhibit IFN-γ production by lectin-stimulated spleen cells. HeLa cells were infected with AdRSVhIL-10 or AdRSV-LacZ, and the CM were collected after 24 h. CM from AdRSVhIL-10–infected HeLa cells was able to inhibit IFN-γ production by approximately 26% compared with that in nontransfected cells (Figure 4). Neither CM from HeLa cells infected with AdRSVLacZ nor a 1:50 dilution of CM from AdRSVhIL-10–infected cells inhibited IFN-γ production. Thus, AdRSVhIL-10 is bioactive *in vitro*, and inhibits IFN-γ expression in lectin-stimulated murine spleen cells in a dose-dependent manner.

In Vivo Bioactivity of AdRSVhIL-10 and Effects of Gene Transfer on HP

To test the bioactivity of AdRSVIL-10 *in vivo*, we administered 4×10^8 pfu of AdRSVhIL-10 or AdRSVLacZ intravenously to IL-10 KO mice. Human IL-10 levels in sera were measured at Day 6. On Day 9, 10 μg of LPS was ad-

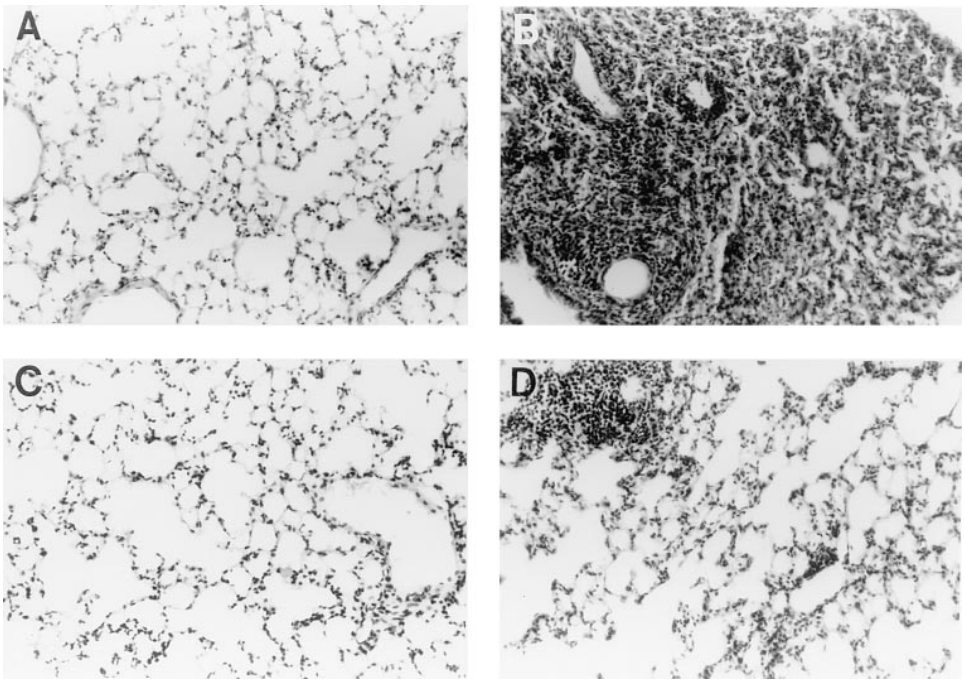


Figure 2. Expression of hypersensitivity pneumonitis in IL-10 KO and WT mice. (*A*) IL-10 KO mice exposed to saline alone. (*B*) IL-10 KO mice exposed to antigen. (*C*) WT mice exposed to saline alone. (*D*) WT mice exposed to antigen. Representative H&E-stained histology sections. Magnification: ×160.

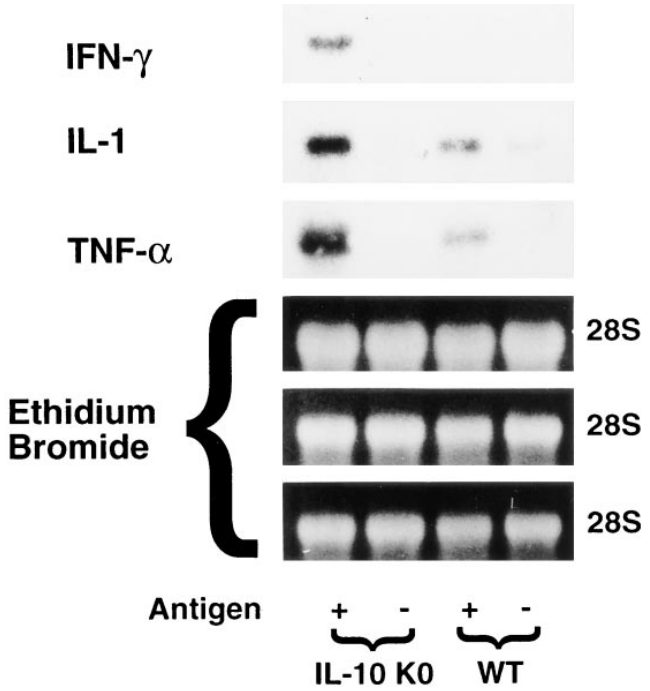


Figure 3. Northern blot analysis for IFN-γ, IL-1, and TNF-α mRNAs. Both IL-10 and WT mice showed increased production of TNF and IL-1 mRNA when exposed to antigen, but IFN-γ mRNA could be detected only in IL-10 KO mice. Ethidium bromide staining demonstrates equal loading of RNA ($n = 3$).

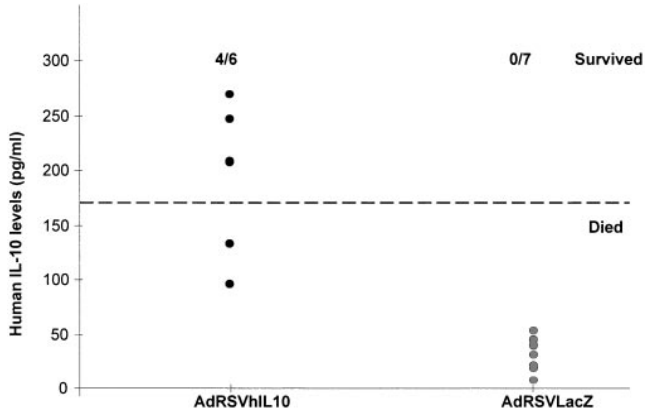
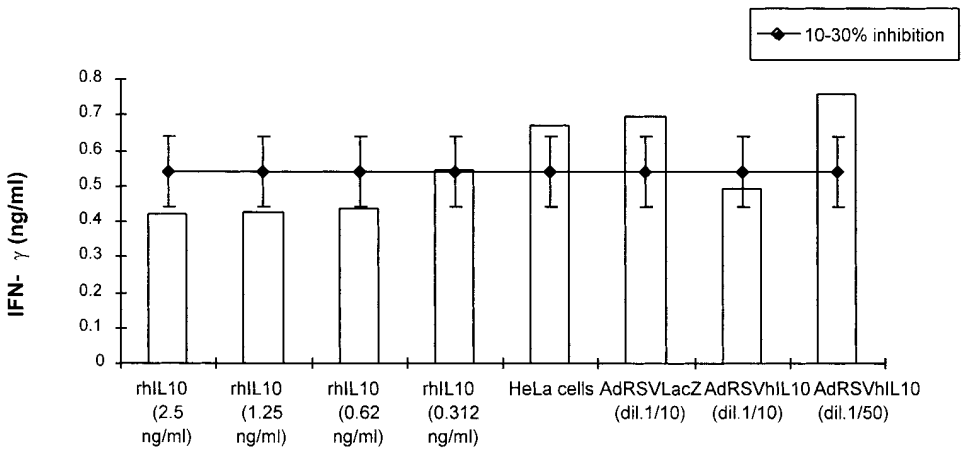


Figure 5. AdRSVhIL-10 gene transfer protects IL-10 KO mice from LPS toxicity. IL-10 KO mice were given AdRSVhIL-10 or AdRSVLacZ, and hIL-10 in sera was measured 1 wk later. All mice were challenged intravenously with 10 μg LPS and monitored for endotoxemia. The level of circulating hIL-10 required for survival is indicated. The level detected in AdRSVLacZ-treated mice came from background cross-reactivity. Data are expressed as means + SEM for $n = 6$ animals for the AdRSVhIL-10 group and $n = 7$ animals for the AdRSVLacZ group.

ministered intravenously, and the animals' survival was monitored for the next 72 h (Figure 5). Normal mice of the same genetic background were not affected by this amount of LPS (data not shown). All IL-10 KO mice treated with AdRSVLacZ succumbed to the endotoxin, whereas four of six mice treated with AdRSVhIL-10 survived. The levels of hIL-10 found in sera show that 200 pg/ml of hIL-10 was able to protect IL-10 KO mice from death caused by LPS.

In separate experiments, we replaced IL-10 through adenovirus-mediated gene transfer to the livers of IL-10 KO mice prior to induction of HP, to confirm the role of IL-10 in HP. Mice that received AdRSVIL-10 had 48 ± 16 ng/ml of hIL-10 in their blood at 7 d after injection of the viral vector. In these experiments, no hIL-10 was detected in AdRSVLacZ-injected animals (data not shown). Intranasal injections with SR or saline were initiated at this time. Upon killing of animals, histologic analysis revealed that IL-10 KO mice given AdRSVIL-10 had less granulomatous inflammation (46.6%, mean − SEM = 41.3%, mean + SEM = 52.0%) than did mice that received the control adenovirus vector AdRSVLacZ (66.5%, mean − SEM = 62.6%, mean + SEM = 70.2%), as shown in Figure 6 ($P < 0.0001$). This suggests that the more severe inflammatory response to antigen seen in IL-10 KO mice was due to the absence of IL-10.

Figure 4. In vitro bioassay for hIL-10 after AdRSVhIL-10 infection of HeLa cells. IFN-γ was measured after exposure of murine splenocytes to Con A. The addition of conditioned media from mock-, AdRSVhIL-10-, or AdRSVLacZ-transduced HeLa cells was tested for its ability to inhibit IFN-γ production. The effects of adding increasing doses of purified recombinant hIL-10 are also shown. The concentrations and dilutions of conditioned media used are indicated. Bioactivity is defined as a 10 to 30% inhibition of the production of IFN-γ.



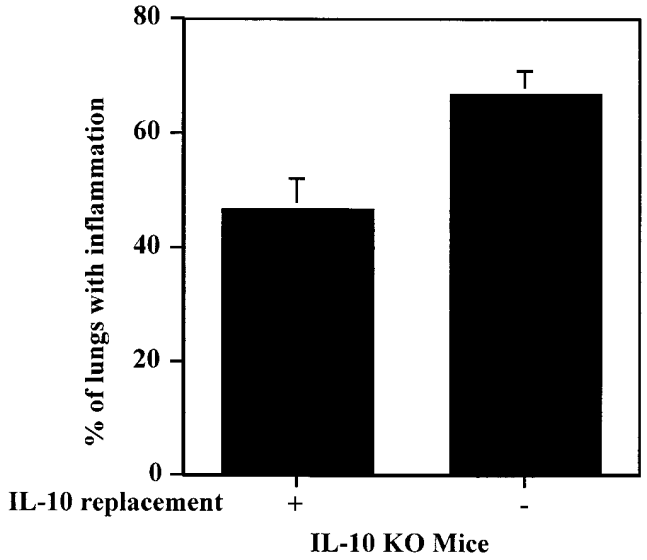


Figure 6. Quantitation of inflammation and granulomas in IL-10 KO mice with and without IL-10 replacement therapy. IL-10 KO mice were exposed to antigen intranasally, and AdRSVhIL-10 or AdRSVLacZ was administered intravenously. The amounts of inflammation and granulomas were quantitated for both groups. Antigen-exposed IL-10 KO mice had significantly more inflammation than antigen-exposed IL-10 KO mice that were given IL-10 replacement therapy ($P < 0.0001$). Data are expressed as the means ± SEM for $n = 4$ animals in each group.

Discussion

The goal of this study was to evaluate the role of IL-10 in the pathogenesis of HP. To address this question, we compared the development of HP in mice that express the IL-10 gene with that in mice of the same genetic background (C57BL/6) that cannot express this gene. We found that when IL-10 KO mice were exposed to antigen, they accumulated more inflammatory cells in their BALF and had greater histologic evidence of inflammation and granuloma formation than did WT mice. IFN-γ, TNF-α, and IL-1 have been shown to mediate the development of granulomas in other models of granulomatous disease (25–31). The IL-10 KO mice also expressed higher levels of IFN-γ, TNF-α, and IL-1 mRNA in their lungs after exposure to antigen. These observations suggest that IL-10 modulates, at least in part, the severity of HP by dampening the upregulation of IFN-γ, TNF-α, and IL-1 genes in lung.

In a murine model of allergic bronchopulmonary aspergillosis, Grunig and associates (41) also found that IL-10 suppresses cytokine production and inflammation. Outbred IL-10 KO mice exposed to *Aspergillus fumigatus* developed a neutrophilic response in their BALF, with high levels of IFN-γ and IL-5 in the BALF and increased inflammation as documented by histologic evaluation, compared with WT mice. However, IL-10 KO mice of a C57BL/6 background had similar amounts of inflammation to those of WT mice with an eosinophilic response in their BALF, and no differences were found in IFN-γ. These studies, although in a different disease model than ours, support the findings of the current study.

Macrophages and lymphocytes are important cellular mediators of the granulomatous inflammatory response in lung. These cells produce several proinflammatory cytokines, including IL-1, TNF-α, and IFN-γ. We have previously shown that IFN-γ is essential for granuloma formation in HP, and that mice that lack production of IFN-γ do not form granulomas when exposed to antigen (21). That study and others (27, 28, 30) suggested that IFN-γ, TNF-α, and IL-1 are important for the cellular immune responses leading to granuloma formation. In studies of both humans and mice, the initial response to antigen in alveoli and small airways is an increase in PMN (12, 16). Later, lymphocytes become more prominent. The present study shows that a prominent neutrophil response was still evident 4 d after exposure to SA antigen in IL-10 KO mice. This was not seen in WT mice given the same dose of SR antigen, and demonstrates that IL-10 is important for cellular responses in HP.

IL-10 is produced by many different cell types, including B and T lymphocytes and macrophages, and affects both those cells and others, such as neutrophils (42). In addition to inhibiting the proinflammatory cytokines IFN-γ, TNF-α, and IL-1, IL-10 also inhibits the production of chemokines that are powerful chemoattractants for neutrophils (43). As a consequence, IL-10 KO mice have a more prominent neutrophil response to antigen. The sustained neutrophil response in IL-10 KO mice could further magnify the inflammatory response, because neutrophils are important in mononuclear cell recruitment. It is of interest that macrophages both produce and respond to IL-10 (23). It has been proposed that the production of IL-10 by macrophages provides a mechanism whereby these cells limit their own participation and that of other cells in antigen-driven reactions (44).

To evaluate whether the changes seen in the IL-10 KO mice were due to lack of expression of the deleted gene or were a result of a compensatory developmental process, hIL-10 was provided by adenovirus-mediated gene transfer to the liver. This route was chosen because it was previously well established as producing detectable levels of hIL-10 in serum over the time period during which intranasal injections of SR antigen take place in this murine model of HP. This resulted in decreased inflammation in these animals upon exposure to antigen, and suggests that the increased inflammatory response to antigen in IL-10 KO mice is due to lack of this important cytokine. The observations in this study show that the IL-10 dampens the inflammatory response in a murine model of HP. These results further suggest that the IL-10 response to antigen inhalation is important in the expression of disease in patients with HP. It is possible that individuals who are resistant to developing HP have prominent IL-10 responses that help to dampen the proinflammatory response that occurs with inhalation of the organic antigens that cause HP. These studies also suggest that IL-10 can be used to treat patients with HP. Further studies of gene therapy with IL-10, not only with hepatic expression but also with intratracheal expression, might be warranted in some patients with this disease.

Acknowledgments: Supported in part by a VA Merit Grant, and Grants HL-37121 and AI35018 from the National Institutes of Health. Dr. Gudmundsson is

funded in part by a Research Fellowship Grant from the American Lung Association of Iowa, a NATO Science Fellowship, a Research Fellowship Grant from the American Heart Association, Iowa affiliate, and the National Institute for Environmental Health Sciences Research Center Grant NIEHS/NIH P30 ESO5605. Dr. Davidson is a fellow of the Roy J. Carver Trust.

The authors thank Anita Riggan for outstanding secretarial assistance and Jannell Pemberton for technical assistance. They also acknowledge the University of Iowa Gene Transfer Vector Core, supported in part by the Carver Foundation, for providing purified viral stocks.

References

1. Fink, J. N. 1992. Hypersensitivity pneumonitis. *Clin. Chest Med*. 13:303–309.
2. Sharma, O. P., and N. Fujimura. 1995. Hypersensitivity pneumonitis: a non-infectious granulomatosis. *Semin. Respir. Infect.* 10:96–106.
3. Krasnick, J., H. J. Meuwissen, M. A. Nakao, A. Yeldandi, and R. Patterson. 1996. Hypersensitivity pneumonitis: problems in diagnosis. *J. Allergy Clin. Immunol*. 97:1027–1030.
4. Gurney, J. W. 1988. Hypersensitivity pneumonitis: correlation of cellular and immunologic changes with clinical phases of disease. *Lung* 166:189–208.
5. Salvaggio, J. E. 1994. Inhaled particles and respiratory disease. *J. Allergy Clin. Immunol*. 94:304–309.
6. Salvaggio, J. E. 1990. Recent advances in the pathogenesis of allergic alveolitis. *Clin. Exp. Allergy* 20:137–144.
7. Salvaggio, J. E., and B. W. Millhollon. 1993. Allergic alveolitis: new insights into old mysteries. *Respir. Med*. 87:495–501.
8. do Pico, G. A. 1992. Hazardous exposure and lung disease among farm workers. *Clin. Chest Med*. 13:311–328.
9. Pepys, J., R. Riddell, and K. M. Citron. 1962. Precipitins against hay and moulds in the serum of patients with farmer's lung aspergillosis, asthma and sarcoidosis. *Thorax* 17:366–374.
10. Keller, R. H., J. N. Fink, and S. Lyman. 1982. Immunoregulation in hypersensitivity pneumonitis: 1. Differences in T-cell and macrophage suppressor activity in symptomatic and asymptomatic pigeon breeders. *J. Clin. Immunol.* 2:46–54.
11. Semenzato, G., R. Zambello, L. Trentin, and C. Agostini. 1993. Cellular immunity in sarcoidosis and hypersensitivity pneumonitis. Recent advances. *Chest* 103:139S–143S.
12. Fournier, E., A. B. Tonnel, P. H. Gosset, B. Wallaert, J. C. Ameisen, and C. Voisin. 1985. Early neutrophil alveolitis after antigen inhalation in hypersensitivity pneumonitis. *Chest* 88:563–566.
13. Drent, M., H. van Velzen-Blad, M. Diamant, S. S. Wagenaar, H. C. Hoogsteden, and J. M. van den Bosch. 1993. Bronchoalveolar lavage in extrinsic allergic alveolitis: effect of time elapsed since antigen exposure. *Eur. Respir. J.* 6:1276–1281.
14. Cormier, Y., J. Belanger, and A. Tardif. 1986. Relationship between radiographic change, pulmonary function, and bronchoalveolar lymphocytosis in farmer's lung disease. *Thorax* 41:28–33.
15. Jagerroos, H., A. V. Seppa, and R. A. Mantyjarvi. 1986. Pulmonary and immune responses to a *Thermoactinomyces vulgaris* antigen respiratory sensitization in C57BL/6 mice. *Exp. Pathol*. 29:95–102.
16. Takizawa, H., M. Suko, N. Kobayashi, S. Shoji, K. Ohta, M. Nogami, H. Okudaira, T. Miyamoto, and J. Shiga. 1988. Experimental hypersensitivity pneumonitis in the mouse: histologic and immunologic features and their modulation with cyclosporin A. *J. Allergy Clin. Immunol.* 81:391–400.
17. Takizawa, H., K. Ohta, T. Horiuchi, N. Suzuki, T. Ueda, M. Yamaguchi, N. Yamashita, A. Ishii, M. Suko, and H. Okudaira. 1992. Hypersensitivity pneumonitis in athymic nude mice: additional evidence of T cell dependency. *Am. Rev. Respir. Dis*. 146:479–484.
18. Schuyler, M., K. Gott, A. Cherne, and B. Edwards. 1997. Th1 CD4+ cells adoptively transfer experimental hypersensitivity pneumonitis. *Cell Immunol.* 177:169–175.
19. Denis, M., Y. Cormier, M. Laviolette, and E. Ghadirian. 1992. T cells in hypersensitivity pneumonitis: effects of *in vivo* depletion of T cells in a mouse model. *Am. J. Respir. Cell Mol. Biol*. 6:183–189.
20. Denis, M., Y. Cormier, and M. Laviolette. 1992. Murine hypersensitivity pneumonitis: a study of cellular infiltrates and cytokine production and its modulation by cyclosporin A. *Am. J. Respir. Cell Mol. Biol.* 6:68–74.
21. Gudmundsson, G., and G. W. Hunninghake. 1997. Interferon-gamma is necessary for expression of hypersensitivity pneumonitis. *J. Clin. Invest.* 99:2386–2390.
22. Fiorentino, D. F., M. W. Bond, and T. R. Mosman. 1989. Two types of murine helper T cell: IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med*. 170:2081–2095.
23. de Waal Malefyt, R., J. Abrams, B. Bennet, C. G. Figdor, and J. E. de Vries. 1991. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med*. 174:1209–1220.
24. Miethke, T., C. Wahl, K. Heeg, B. Echtenacher, P. H. Krammer, and H. Wagner. 1992. T cell-mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumour necrosis factor. *J. Exp. Med*. 175:91–98.
25. Tsuji, M., V. B. Dimov, and T. Yoshida. 1995. *In vivo* expression of monokine and inducible nitric oxide synthase in experimentally induced pulmonary granulomatous inflammation: evidence for sequential production of interleukin-1, inducible nitric oxide synthase, and tumor necrosis factor. *Am. J. Pathol*. 147:1001–1015.
26. Flory, C. M., M. L. Jones, B. F. Miller, and J. S. Warren. 1995. Regulatory roles of tumor necrosis factor-alpha and interleukin-1 beta in monocyte chemoattractant protein-1-mediated pulmonary granuloma formation in the rat. *Am. J. Pathol*. 146:450–462.
27. Chensue, S. W., K. S. Warmington, J. H. Ruth, P. Lincoln, and S. L. Kunkel. 1995. Cytokine function during mycobacterial and schistosomal antigen-induced pulmonary granuloma formation. Local and regional participation of IFN-gamma, IL-10, and TNF. *J. Immunol*. 154:5969–5976.
28. Chensue, S. W., K. Warmington, J. Ruth, P. Lincoln, M. C. Kuo, and S. L. Kunkel. 1994. Cytokine responses during mycobacterial and schistosomal antigen-induced pulmonary granuloma formation. Production of Th1 and Th2 cytokines and relative contribution of tumor necrosis factor. *Am. J. Pathol*. 145:1105–1113.
29. Kasama, T., K. Kobayashi, N. Yamagata, H. Iwabuchi, T. Matsuda, K. Nakatani, K. Kasahara, and T. Takahashi. 1992. Augmentation of pulmonary foreign body granulomatous inflammation in mice by lipopolysaccharide: involvement of macrophage activation and tumor necrosis factor-alpha. *Int. Arch. Allergy Immunol*. 97:130–138.
30. Denis, M., and E. Ghadirian. 1992. Murine hypersensitivity pneumonitis: bidirectional role of interferon-gamma. *Clin. Exp. Allergy* 22:783–792.
31. Denis, M., Y. Cormier, M. Fournier, J. Tardif, and M. Laviolette. 1991. Tumor necrosis factor plays an essential role in determining hypersensitivity pneumonitis in a mouse model. *Am. J. Respir. Cell Mol. Biol.* 5:477–483.
32. Kuhn, R., J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75:263–274.
33. Clark, D. 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, DC.
34. Holt, P. G., J. E. Batty, and K. J. Turner. 1981. Inhibition of specific IgE responses in mice by preexposure to inhaled antigens. *Immunology* 42:409–417.
35. Chirgwin, J. M., A. E. Pryzbala, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294–5299.
36. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Extraction, purification, and analysis of messenger RNA from eukaryotic cells. *In* Molecular Cloning: A Laboratory Manual. C. Nolan, editor. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 7.1–7.8.
37. Lehrach, D., D. Diamond, J. Wosney, and H. Boedtker. 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry* 16:4743–4751.
38. Davidson, B. L., S. E. Doran, D. S. Shewach, J. M. Latta, J. W. Hartman, and B. J. Roessler. 1994. Expression of *Escherichia coli* β-galactosidase and rat HPRT in the CNS of *Macaca mulatta* following adenoviral mediated gene transfer. *Exp. Neurol.* 125:258–267.
39. Mossmann, T. 1994. Measurement of mouse and human interleukin-10. *In* Current Protocols in Immunology. J. E. Coligan, A. M. Kruisbeck, D. H. Margulies, E. M. Shevach, and W. Strober, editors. Current Protocols, New York.
40. Berg, D. J., R. Kuhn, K. Rajewsky, W. Muller, S. Menon, N. Davidson, G. Grunig, and D. Rennick. 1995. Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance. *J. Clin. Invest.* 96:2339–2347.
41. Grunig, G., D. B. Corry, M. W. Leach, B. W. P. Seymour, V. P. Kurup, and D. M. Rennick. 1997. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J. Exp. Med*. 185:1089–1099.
42. de Waal Malefyt, R., H. Yssel, M. G. Roncarolo, H. Spits, and J. E. de Vries. 1992. Interleukin-10. *Curr. Opin. Immunol.* 4:314–320.
43. Haelens, A., A. Wuyts, P. Proost, S. Struyf, G. Opdenakker, and J. van Damme. 1996. Leukocyte migration and activation by murine chemokines. *Immunobiology* 195:499–521.
44. Rennick, D., N. Davidson, and D. Berg. 1995. Interleukin-10 gene knockout mice: a model of chronic inflammation. *Clin. Immunol. Immpathol.* 76: S174–S178.